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BRINED CHERRIES
Analytical and Quality Control Methods

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Analytical and Quality Control Methods

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In researches on brined cherry technology, a number of physical and chemical tests have been used in studies of certain phases of the complex changes occurring in cherries held in calcium bisulfite solutions. Previous reports noted some of these techniques (5, 7).

Normally, sweet cherries become bleached and firm to the touch after several weeks in the bisulfite brines commonly used. Occasionally they become soft, however, and disintegrate so that they cannot be processed into marachino cherries or fruit for cocktail or other products. This unusual softening has been the subject of research, and many of the methods detailed below are applicable to determining texture or factors which might influence texture.

In the industry there are different methods of formulating calcium bisulfite brines as follows: (1) bubbling sulfur dioxide gas into a suspension of calcium hydroxide; (2) bubbling sulfur dioxide gas into a suspension of calcium carbonate; (3) dissolving calcium chloride and sodium bisulfite in water and adjusting pH with commercial hydrochloric acid. Listed below are formulations which have been widely used and can be expected to give satisfactory brined cherries with fruit produced in the western States. Individual briners occasionally employ modifications of these formulations for certain lots of fruit. Commercial grades of the chemicals are not 100 percent pure; amounts used for making brine must be adjusted according to the composition given on the label. The formulations follow:

(1) Add commercial hydrated lime ($Ca(OH)_2$) to water at the rate of 6 lb. per 100 gal. Stir well to form a suspension. Introduce sulfur dioxide gas into the lime slurry by means of a perforated tube or other submerged bubbling device. Dissolve 10.5 lb. of sulfur dioxide. The brine will turn nearly clear when the proper amount of sulfur dioxide has been dissolved. The pH of this brine will be about 2.7 ± .2. Check the sulfur dioxide and calcium contents by methods described under "Analytical methods."

(2) Add commercial whiting ($CaCO_3$) to water at the rate of 8 lb. per 100 gal. Stir

well to form a suspension. Introduce sulfur dioxide gas into the slurry of whiting by means of a perforated stainless-steel tube or other submerged bubbling device. Dissolve 10.5 lb. sulfur dioxide per 100 gal. to give a solution containing about 1.25 percent of sulfur dioxide. During the addition of sulfur dioxide gas, considerable bubbling will usually take place due to the formation of carbonic acid and evolution of carbon dioxide. Loss of sulfur dioxide may be minimized by keeping a floating lid on the surface of the tank in which the brine is made. The brine will turn nearly clear when the proper amount of sulfur dioxide is dissolved. The pH of this brine will be 2.0 ±.2. Check the sulfur dioxide and calcium contents by methods described under "Analytical methods."

(3) Add 14 lb. of anhydrous sodium bisulfite ($NaHSO_3$) or 12.5 lb. of anhydrous sodium metabisulfite ($Na_2S_2O_5$) to 100 gal. of water with stirring. Add 5 fluid oz. of commercial hydrochloric acid. Add 7 lb. of commercial anhydrous calcium chloride and adjust the acidity to about pH 3.5 by adding more acid. This brine will contain about 1 percent sulfur dioxide and 0.85 percent calcium chloride (or 0.3 percent calcium ion). Check the sulfur dioxide and calcium levels by methods given in "Analytical methods." If the ingredients are mixed in a different order from that indicated here, the solution may become very cloudy and much insoluble material may be precipitated.

CONTROL SAMPLING

Sampling devices.--Cherries can be withdrawn from a barrel through the bung by means of a stout wire bent at an acute angle 2 or 3 in. from one end. The stems of cherries are caught in the sharp bend and they can then be lifted out through the bung. A more satisfactory method is to replace the head of a sample barrel with a plastic or heavy-paper sheet. This can be easily removed for withdrawing samples.

Samples from a tank containing several thousand gallons can be removed by the device shown in figure 1. The pronged device is thrust into the tank to the desired sampling point, then pulled out. The cherries are caught between the prongs. Sampling devices should be rinsed in clean water before use and between samplings of different containers.

Samples of brine can be withdrawn by means of small-bore pipes or glass tubes.

Procedure and schedule.--Eighteen cherries is a satisfactory sample for estimating the texture of cherries in a barrel (1). The number of barrels to be sampled in each lot of fruit will depend on many factors and will not be specified here. It would be well to sample lots or tanks at 2-week intervals during the first 6 months after brining. During the first week in brine, the cherries become engorged with liquid and appear soft; then they begin to become firm and their texture becomes firmer for several weeks.

A large tank of brined cherries may be considered as a composite of barrels and sampled accordingly. A 10,000-gal. tank could be visualized as containing 200 bbl. (50 gal. each) of brined cherries. If each 20 bbl. are to be sampled at one time,

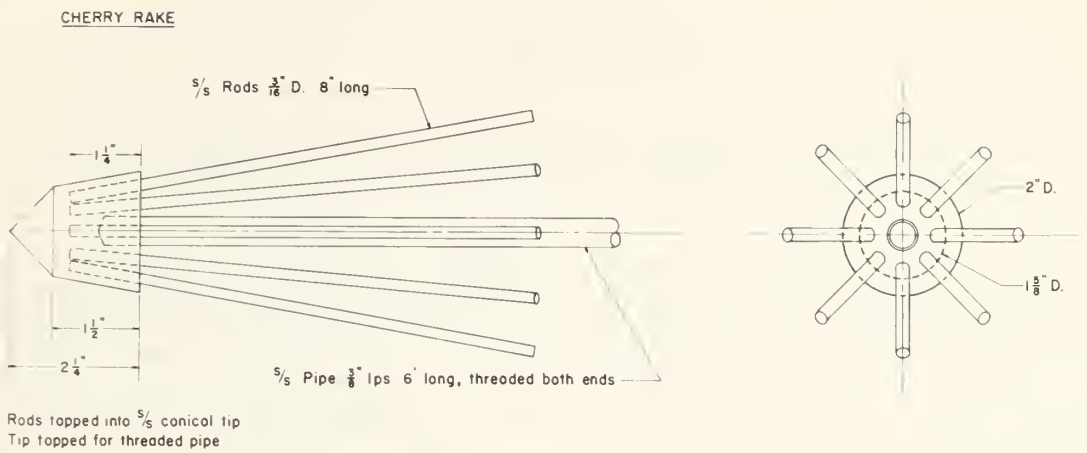

Figure 1. --Scale drawing of cherry sampling device (cherry rake)

10 sub-samples of 18 cherries each can be withdrawn from scattered points in the tank. The texture of the cherries is measured by means of the puncture meter described in "Texture measurements."

Brine samples and cherry samples can be taken at the same time. The calcium ion, sulfur dioxide, pH, and enzyme tests can all be run on a 100-ml. sample.

ANALYTICAL METHODS

Many procedures described here are long-accepted, standard methods of analysis, some with minor modifications which enhance their usefulness on brined cherries. Some are the result of new ideas developed into test methods.

Sulfur Dioxide

This is the method of Ponting and Johnson (4). The reagents used are 1 percent starch indicator solution and 0.1N iodine solution.

To 20 ml. of distilled water in a 125-ml. Erlenmeyer flask, add 5.0 ml. of brine and 1 ml. of 1 percent starch indicator solution. Titrate with 0.1N iodine solution to a blue end-point that persists 30 seconds. Multiply the number of milliliters of iodine by .0640 to give percent sulfur dioxide, or by 640 to give p.p.m. free sulfur dioxide.

Calcium: Colorimetric Method

The following procedure, essentially the same as reported by Strachan and Moyls (6), is a direct colorimetric titration of calcium with a standard versenate solution. A carbonyl reagent is added to eliminate interference by anthocyanins. The indicator produces an orange-red or salmon-pink color in the buffered sample (pH 12.5) containing calcium. When enough versenate is added, the calcium is completely held in complex and the indicator changes color to a violet-blue or orchid-purple. The reagents used are:

Standard (0.01M) versenate solution: Dissolve 4 g. of disodium dihydrogen versenate (disodium dihydrogen ethylenediamine tetraacetate) in approximately 800 ml. of distilled water. If the pH is not between 4.25 and 5.0, adjust by adding reagent grade sodium hydroxide solution (1M) and make up to 1 liter of solution. One ml. of this standard solution is equivalent to 1 mg. of calcium carbonate or 0.4 mg. of calcium.

Primary standard calcium chloride solution: Exactly 1 g. of pure calcite or primary standard grade calcium carbonate is dissolved in the minimum quantity of dilute hydrochloric acid, boiled, and cooled. After cooling it is made up to 1 liter with distilled water. One ml. of this solution equals 1 mg. of calcium carbonate, or 0.4 mg. of calcium and is used to standardize the versenate titrating solution.

Alkaline solution (1N sodium hydroxide solution).

Calcium indicator: Mix 0.2 g. of ammonium purpurate (murexide) with 100 g. of reagent grade sodium chloride. Grind the mixture to 20-mesh. This dye is unstable in most solutions but keeps well in a dry mixture.

Hydroxylamine (0.5M solution of hydroxylamine hydrochloride): Dissolve 35 g. in 1 liter of water.

Test procedure for brine: If the brine is cloudy, filter through coarse filter paper to remove sediment.

Transfer 10 ml. of 0.5M hydroxylamine hydrochloride to a 250-ml. volumetric flask containing 190 ml. of distilled water. Pipette 20 ml. of cherry brine into the flask and bring to volume with 1N sodium hydroxide. The pH should be about 12.5; if not, adjust by adding sodium hydroxide or hydrochloric acid. The solution will turn blue on addition of sodium hydroxide but changes to yellow in about 15 minutes or less. After the solution turns yellow, pipette 50 ml. into a 250-ml. Erlenmeyer flask. Add 50 ml. of distilled water and about 0.4 g. of indicator (murexide). The color will turn salmon-orange if calcium is present. Titrate with 0.01M versenate solution to a purple tinge. The end-point is a final change to violet-blue or orchid-purple.

For fresh brines, pipette into an Erlenmeyer flask or evaporating dish the size sample that will require 10 to 20 ml. of standardized versenate solution. Adjust to pH 12.5 with 1N sodium hydroxide and proceed as above.

Calculate as follows:

$$\text{p.p.m. calcium} = \frac{\text{ml. versenate} \times 400}{\text{ml. of sample}}$$

or

$$\text{p.p.m. as calcium carbonate} = \frac{\text{ml. versenate} \times 1000}{\text{ml. of sample}}$$

Note that a white precipitate or opacity sometimes is formed when the solution is adjusted to pH 12.5. If this is not too dense or voluminous, the end-point is still perceptible.

When the dye-indicator is first added, the solution may remain colorless until it has been shaken for several seconds.

Determining pH

An electrically operated glass electrode pH meter is satisfactory for measuring pH. Instructions and precautions for correct operation are provided with the instrument. The electrode assembly must be kept clean and thoroughly rinsed between samples. The instrument should be standardized against a fresh saturated solution of potassium acid tartrate; this solution, pH 3.57 at 25° C. is near the range of pH found in most satisfactory cherry brines. Kits for colorimetric estimation of pH are also available and may be useful for measuring pH of brine.

Texture Measurements

An objective measurement of the texture (firmness) of brined cherries can be made by a simple puncture meter. Figure 2 shows a Chatillon spring push gauge, 0-500 g., with an L. S. Starrett Co. Vise "B" and fitted with a stainless steel tip 0.08 in. in diameter. The sliding indicator clip "A" is slightly modified from the one provided with the meter by the manufacturer. Figure 3 is a scale drawing of the modified sliding clip.

A puncture test is done by holding a cherry in one hand and pressing the tip of the puncture meter against its side. The puncture meter is held by the barrel between points "A" and "C" indicated on figure 2. As force is exerted against the cherry, the slider "A" is pushed up along the guide rod. When the tip breaks through the tissue, the maximum reading in grams of force is indicated on the scale opposite the slider "A". About 15 to 20 cherries can be punctured per minute. The meter is easily calibrated by pressing the tip against one pan of a balance and adjusting collar "C" so that the meter gives its reading in grams.

The texture of the cherries in a barrel can be estimated by puncture tests on a sample of 18 cherries; the average and standard deviation both provide useful information about the texture of the brined fruit. Calculate the average texture value for each sample of cherries. The standard deviation (s) for this set of values can be calculated

Figure 2. --Puncture Meter.

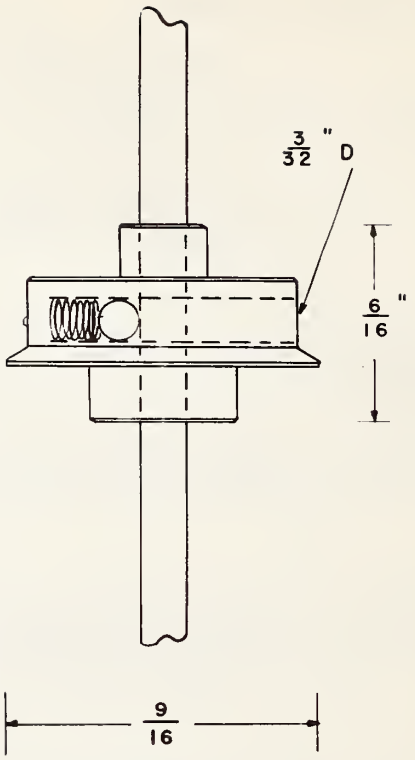

Figure 3. --Scale drawing of modified sliding indicator on puncture meter.

by using the formula:

$$\text{Standard deviation (s)} = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{18}}{17}}$$

ΣX^2 = sum of the squares of individual puncture meter readings.

$(\Sigma X)^2$ = square of the sum of all puncture meter readings.

X = individual puncture meter reading.

After the second week in brine, the average texture reading on normal firm cherries can be expected to be over 250 g.; the standard deviation of the measurements can be expected to be 50 g. or below. If the cherries are becoming soft, the average texture meter values go down and the standard deviation for the sample of 18 cherries will be 70 or higher. If the standard deviation exceeds 70, the onset of an undesirable change in the texture (softening) may be suspected and remedial action should be taken. Either more calcium chloride ($CaCl_2$) should be added, or the cherries should be processed to the end product desired. About 10 lb. of calcium chloride should be added to

each 100 gal. of brined cherries in order to raise the calcium content to about four times the original level. This will retard the softening of cherries in many instances.

Enzymes

The role of enzymes in the softening of brined cherries is not clearly defined. However, model systems in which certain enzymes have been added to brined cherries have demonstrated that softening can be induced in this way. In some samples of cherries that were softening "naturally" (no enzyme added), pectinolytic enzyme activity could be detected. If a high level of pectinolytic enzyme activity were to be measured in a sample of brined cherries, it would likely be accompanied or followed by a substantial decrease in texture or firmness. Enzyme tests and texture measurements together can provide information useful in predicting the onset of softening. With this information at hand, a processor can process the brine-cured cherries before softening proceeds to the point where the fruit cannot be used.

Pectinolytic enzyme: Cup Plate.--This is the method of Dingle et al.(2). A thousand milliliters of medium is prepared as follows:

1. To 500 ml. distilled water in a 1-qt. blender jar, add 2.50 g. of polygalacturonic acid (available from Sunkist as No. 491).

2. While blending add 75 ml. of 0.1N sodium hydroxide. (Check pH, which should be 4.0 $\pm$0.1.)

3. Add 2.5 g. ammonium oxalate and 10.0 g. potassium acid phthalate. Continue to blend until completely dissolved.

4. Transfer to 1-liter Erlenmeyer.

5. Blend 20.0 g. Bacto Agar into 425 ml. of distilled water and add 0.25 g. thymol.

6. Transfer to a second 1-liter Erlenmeyer.

7. Steam both flasks about 30 minutes or until dissolved. (Agar will become clear above 97° C.)

8. Cool contents of both flasks to 50° C.; mix in a 2-liter flask.

9. Dispense to flat-bottomed petri dishes, 25 ml. per dish.

Small stainless steel collars (8 mm. dia.) may be imbedded in the media to provide cups into which a few drops of brine can be placed (Fig. 4). The collars (1/4 in. long) can be cut from stainless steel tubing.

With an eye dropper or similar device, dispense 4 or 5 drops of test sample into a cup. Incubate overnight at 35° C. At the end of the incubation period, pour 6N hydrochloric acid (commercial hydrochloric acid diluted about 50 percent) over the media in the plates. Allow to stand 1 hour and observe. If polygalacturonase is present, a clear zone will appear around the cup (Fig. 5); the diameter will vary depending

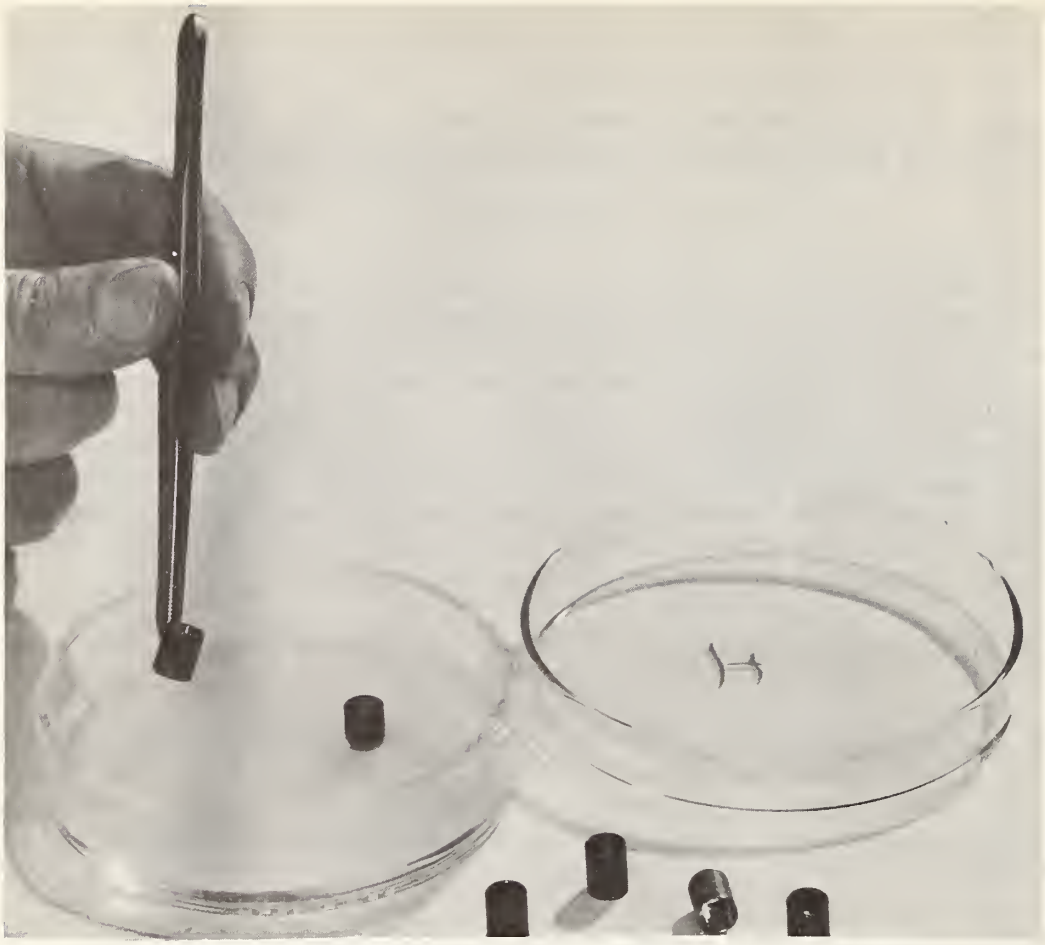

Figure 4.--Assembly for cup-plate test showing petri dish containing media and stainless steel collars that form the cups for brines or solutions to be tested.

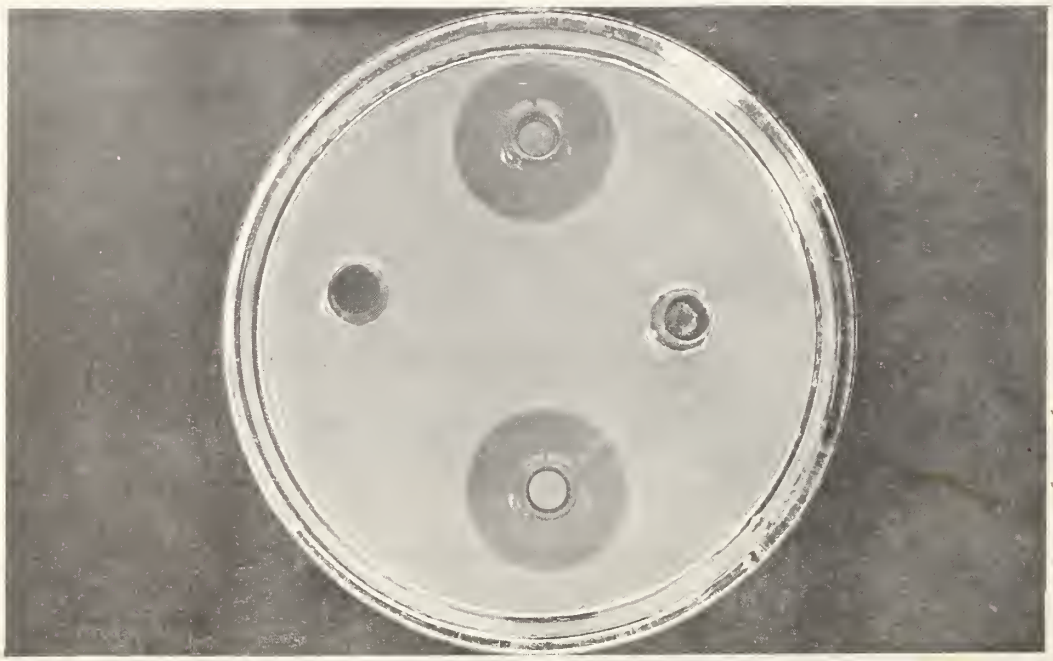

Figure 5.--Cup-plate test after incubation and development with 6N HCl. Note clear zones caused by polygalacturonase activity.

on the concentration of the enzyme.

Polygalacturonase activity is evaluated by measuring the diameter of the clear zone, including the diameter of the cup. Control samples of solutions of pectin enzymes in water or brine should be included in any series of tests. Solutions containing 1, 0.1, and 0.01 percent of pectic enzyme will give clear zones of different diameters around the cup. By comparing the sample of cherry brine under test with the enzyme solutions, one can estimate the degree of polygalacturonase activity in terms of concentration of the pectic enzyme used. A clear zone 10 mm. in diameter indicates a strong positive test for polygalacturonase; samples with sufficient pectinolytic enzyme added to soften the cherries experimentally will show a clear zone of 10 mm. diameter or larger.

Pectinolytic enzyme: Viscometric.--The reagent used is a 0.5 percent pectin solution. (Slowly add N.F. pectin to distilled water in blender while stirring slowly, stir until dispersed, and add 4 to 6 drops of toluene to preserve.)

To 10 ml. of 0.5 percent pectin solution, add 10 ml. of brine solution from soft cherries. The solutions must be free of visible particles. The reaction time of 30 minutes begins when the first drop of test solution enters the pectin substrate. Pipette 10 ml. of the mixture into an Ostwald-Cannon-Fenske viscometer, size 100 (Fig. 6). Draintime is measured at the end of the 30-minute reaction period. It is also determined on blanks of heat-inactivated brine and of water. (Heat-inactivated brine is prepared as follows: Place about 20 ml. of cherry brine in a test tube with a loose-fitting cork; stand the test tube in a beaker of boiling water and heat it in this fashion until the brine is at 185-190° F.; cool the brine to room temperature rapidly by placing the test tube in a large beaker of cold water. A precipitate may appear in the heat-inactivated brine. Filter the cooled brine, or gently push a plug of glass wool to the bottom of the test tube and withdraw the test sample from the clear brine above.)

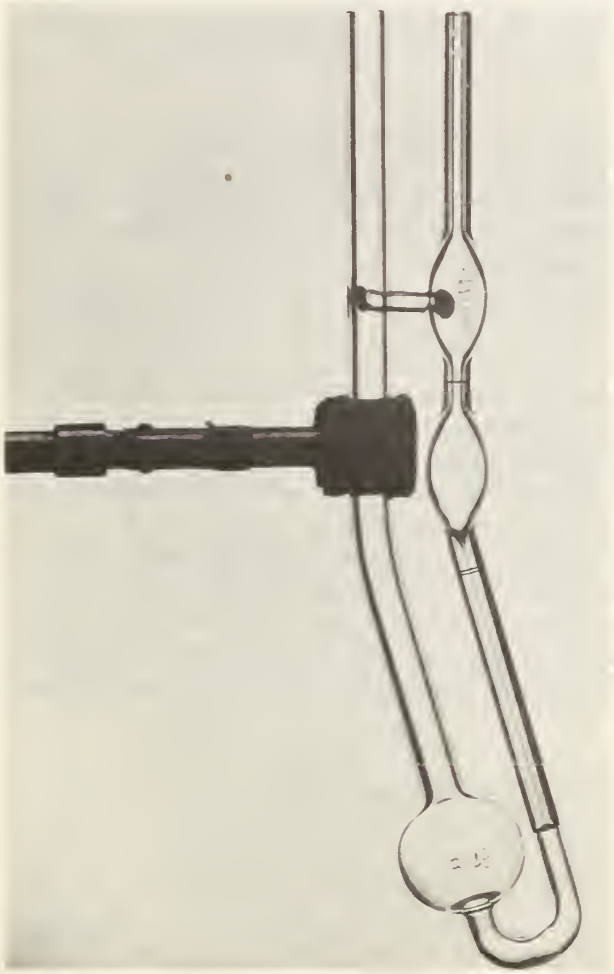

Figure 6.--Viscometer, Ostwald-Cannon-Fenske type, size 100

A = drain time of mixture of heated brine and pectin solution after 30 minutes.

B = drain time of mixture of brine and pectin solution after 30 minutes.

C = drain time of water.

$$\frac{A - B}{A - C} = a_p$$

The symbol a_p is the ratio of differences of drain times which can be used to express activity of agents causing decrease in viscosity of pectin solution. The larger the ratio (a_p), the greater the pectinolytic enzyme activity. This may be taken as an approximation of the activity of a pectin degrading enzyme in the brine. If the brine has substantial pectinesterase activity, the viscosity of the pectin solution will increase and the viscometric test cannot be used.

The a_p value calculated from viscometric estimation of pectinolytic activity can be expected to be 0.05 or less in samples of brine from firm cherries. Brines from soft cherries have shown values of 0.2 and above.

Pectinesterase (Modified method of MacDonnell, Jansen, and Lineweaver (3)). -- The reagents are:

Sodium hydroxide reagent grade - 0.1N and 20 percent solutions,

Pectin substrate - 0.5 percent pectin solution (slowly add N.F. pectin to distilled water in blender while stirring slowly, continue stirring until completely dispersed, and add 4 to 6 drops of toluene for preservation), and

Sodium chloride.

Pipette 50 ml. of brine into a 400-ml. beaker equipped with mechanical stirrer and pH electrodes. Adjust the pH to 7.0 with 20 percent sodium hydroxide and add enough sodium chloride to give a concentration of 0.15M for the total volume. Add 100 ml. of a 0.5 percent pectin solution and adjust the pH to 7.0 with 0.1N sodium hydroxide. During the reaction period of 30 minutes which begins when pH 7.0 is reached, the pH is maintained by titrating the liberated carboxyl groups with 0.1N sodium hydroxide. Blanks containing pectin substrate and brine which has been boiled for 5 minutes are titrated to pH 7.0 and maintained there for the reaction period. Results are expressed as pectinesterase units per milliliter which represent the milliequivalents of ester hydrolyzed per minute per milliliter of brine. The equation used for computing PE units is as follows:

$$\text{PEu/ml.} = \frac{\text{ml. sodium hydroxide x normality}}{\text{ml. of sample x 30}}$$

The significance of pectinesterase in softening of cherries is not established. Fresh cherries have pectinesterase activity which can be expressed as about 1 or 2 PE units per kilogram. The brine from freshly brined cherries may show from 0 to .25 PE units per liter. In some samples of softening cherries PE values of about 0.5 have

been observed. However, the most significant aspect of high pectinesterase values from the standpoint of the analytical methods described here is that a high pectinesterase activity will interfere with the measurement of pectinolytic activity by the viscometric method. It will cause rapid demethylation of the pectin substrate, and calcium ions from the brine in the reaction mixture will combine with liberated carboxyl groups to cause an increase in viscosity or the formation of a gel, if the PE activity is very high.

A widely used method of determining pectinolytic activity involves the measurement of change in the reducing power of the substrate and enzyme mixture. The presence of bisulfites and sugars in cherry brines renders this method extremely difficult to use. Careful fractionation and separation of enzymatically active materials from the brine are necessary before the method can be applied.

Valuable technical assistance was provided by Rogernald Jackson and Doris H. Taylor.

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